

DEVELOPMENTAL
AND FUNCTIONAL
ASPECTS ON
ALPHA—FETOPROTEIN
AND ALBUMIN OF
PORCINE BLOOD
SERUM.



By Bengt Ingvarsson Lund 1979



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DEVELOPMENTAL AND FUNCTIONAL ASPECTS
ON ALPHA-FETOPROTEIN AND ALBUMIN OF
PORCINE BLOOD SERUM

AV

BENGT INGVARSSON

Fil.Kand., Kr.

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Alpha-fetoprotein, AFP, from fetal pig serum and albumin from adult pig serum, fetal pig serum, sow's colostrum and urine from neonatal pigs were isolated by the use of preparative electrophoresis and immunosorbent techniques. The purity of the various protein fractions was ascertained by means of polyacrylamide gel electrophoresis, electroimmunoassay and by immunization of rabbits.

AFP and the various albumins were characterized with respect to molecular weight, sedimentation coefficient, isoelectric pH, amino acid composition and electrophoretic mobility. It was shown that great similarities existed between AFP and albumin in chemical and physicochemical properties.

The serum levels of AFP, albumin and total protein were estimated during the ontogenetic development of the pig. The results show the existence of an inverse relationship between the serum levels of AFP and albumin, with decreasing AFP and increasing albumin levels, except for the first week of extrauterine life when both AFP and albumin increase.

By means of gel electrophoresis and autoradiography it was demonstrated that AFP and albumin bind tryptophan and fatty acids of different lengths and saturation.

The results indicate that AFP may be a fetal counterpart to adult serum albumin as regards the transport of ligands in the blood. The fact that a protein other than albumin shows affinity for several ligands in fetal blood provides a basis for a tentative hypothesis that AFP facilitates the transport of metabolites from the maternal to the fetal circulation via the placenta by having a higher affinity for these substances than albumin

Referat skrivet av

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in maternal blood. Moreover, if maternal albumin has a higher binding affinity for catabolites than AFP, the clearance of catabolites from fetal circulation would be improved.

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BLOOD SERUM

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TILL MOR OCH FAR

TILL EVA , ANNA , JOHAN OCH PER



TO MY ASSISTANT

The present thesis is based upon the following papers:

- I. Carlsson, R.N.K.; Ingvarsson, B.I. and Karlsson, B.W.: Isolation and characterization of alpha-fetoprotein from foetal pigs. Int. J. Biochem. 7:13-20 (1976).
- II. Carlsson, R.N.K.; Ingvarsson, B.I. and Karlsson, B.W.: Isolation and characterization of albumin from porcine serum, colostrum and urine. Int. J. Biochem. 8:285-294 (1977).
- III. Ingvarsson, B.I.; Carlsson, R.N.K. and Karlsson, B.W.: Synthesis of alpha-fetoprotein, albumin and total serum protein in neonatal pigs. Biol. Neonate 34:259-268 (1978).
- IV. Ingvarsson, B.I. and Carlsson, R.N.K.: Binding of fatty acids and tryptophan to alpha-fetoprotein from fetal pigs. Biochim. Biophys. Acta 537:507-509 (1978).

These papers will be referred to by the Roman numerals listed above.

Abbreviations used:

AFP = alpha-fetoprotein

SS = adult swine serum

ASS = antiserum against adult swine serum

CR = crown to rump

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INTRODUCTION

Alpha-fetoprotein, AFP, is a serum protein present in large amounts during the fetal development of mammals (Adinolfi et al., 1975). AFP is mainly synthesized by the yolk sac and the fetal liver (Abelev, 1971). It was first detected in the serum of human fetuses by Bergstrand and Czar (1956). Further investigations have revealed that homologues to mammalian AFP are found in the fetuses of sharks, amphibia, reptiles and birds (Gitlin, 1974), and it has been proposed that AFP has existed as far back in evolution as 400 million years ago, at the time when mammals and elasmobranchs diverged (Gitlin, 1974).

By use of immunological techniques it has been possible to isolate AFP from several animal species (Ruoslahti et al., 1974). The chemical and physicochemical properties of AFP (Ruoslahti et al., 1974; Nishi et al., 1975) as well as serum levels and sites of synthesis (Ruoslahti et al., 1974; Gitlin, 1975) have been investigated extensively. The serum levels of AFP are high during fetal development, with maximum values of 3-10 mg/ml in various mammalian fetuses (Abelev, 1971; Karlsson, 1972; Gitlin, 1975; Lai et al., 1978), and decrease successively during neonatal development (Ruoslahti and Seppälä, 1972; Sell et al., 1974; Gitlin, 1975). In the pig, however, a significant increase occurs in the serum AFP level during the first week of extrauterine life (Karlsson, 1970).

AFP is normally present in low concentrations (1-200 ng/ml) in serum from adult animals (Ruoslahti et al., 1974). However, AFP reappears in serum from adults in connection with hepatocellular malignancies and restorative proliferation (Abelev, 1971). This was first shown by Tatarinov (1964) who observed elevated AFP levels in human sera in patients with primary liver carcinoma. Further studies have revealed

that elevated serum levels of AFP are also found in connection with tumours in the testis and ovary as well as in nonmalignant conditions such as hepatitis and chronic liver diseases (Tomasi, 1977). AFP can be used as a specific diagnostic indicator in order to detect these pathological conditions, and further to study the results of therapy (Alpert et al., 1971). It is thought that the reappearance of this fetal protein results from the decrease of a repressor, which normally appears at the end of embryonic life (Tomasi, 1977). Recently, the detection of significant increased levels of AFP in amniotic fluid and in maternal serum has proved to be of great value in the prenatal diagnosis of fetal malformations, such as neural-tube defects (Lau and Linkins, 1976; Seppälä, 1977).

The fact that AFP is an old protein in terms of phylogenetic history and that it is one of the dominating proteins in fetal serum from various animal species indicate that this protein has important functions during fetal development. This is further supported by the findings that injection of homologous antisera to AFP into pregnant animals induces abortions and fetal abnormalities (Slade, 1973). However, such effects have not been reproduced by others (Sell and Becker, 1978).

The biological role of AFP is still largely unknown. Recent studies have shown that AFP exhibits a specific estrogen binding capacity in some mammalian species (Savu et al., 1974) and that AFP may have immunoregulatory properties (Murgita and Tomasi, 1975a). The physiological significance of these properties has not yet been clearly defined.

It is thus of relevance to investigate if AFP has other specific functions during fetal development. In fact, Abelev (1971) suggested that AFP is a fetal counterpart to albumin. This assumption was based on the fact that AFP and albumin show physicochemical similarities and that AFP dominates in serum during the early period of fetal development

when albumin is only present in trace amounts.

The aim of the research in our laboratory has been partly to study the biological function of AFP as a serum protein during the fetal development of the pig, and partly to study the regulation of AFP synthesis in order to elucidate the mechanisms responsible for the observed neonatal AFP peak.

In the four papers presented here porcine AFP has been studied as a fetal counterpart to serum albumin with respect to biochemical properties (I, II), serum levels during ontogenetic development (III) and ligand binding ability (IV).

MATERIAL

Experimental animals

Pigs of different developmental stages have been used in these investigations. Pig fetuses with CR-lengths of 6-31 cm and adult pigs were obtained from the slaughterhouse at Scanec, Kävlinge. Neonatal pigs were obtained from Svenska Hushållningssällskapet i Malmöhus län försöksgård, Bjärsjölagård. Rabbits were used for the production of antisera.

Collection and preparation of samples

Blood samples from pig fetuses were collected from the umbilical cord or by heart puncture. It was not possible to obtain samples of blood from fetuses with a CR-length of less than 6 cm. Blood samples from neonatal pigs were obtained by ear vein bleeding or by puncture of vena cava cranialis. In control tests it was shown that the blood sampling technique did not affect the protein concentrations presented in paper III. Blood samples from adult pigs (6 months of age) were collected at the slaughterhouse. The blood was allowed to clot at room temperature and serum was prepared by centrifugation.

Colostrum was collected by hand milking sows during farrowing and was defatted by centrifugation.

All samples were frozen at -20°C until further use (within 3 months). No effects of freezing or thawing on AFP or albumin during this period were noted, as revealed by the analytical methods used.

The protein samples were concentrated by use of negative pressure dialysis in collodium bags (cut off 12.000) or by ultrafiltration through an UM 10 diaflo membrane (cut off 10.000).

REVIEW OF METHODS

All the techniques used are fully described in papers I-IV. Therefore only a brief summary is given here.

Isolation procedures

In order to study various basic biochemical characteristics of AFP and albumin and to prepare monospecific antisera for immunohistochemical studies, it was necessary to obtain pure preparations of AFP and albumin.

The isolation procedures for AFP (I) and albumin (II) are combinations of preparative electrophoresis and affinity chromatography.

Analytical methods

Immunoelectrophoresis according to Scheidegger (1955) was performed in order to determine the potency and specificity of the different antisera, and to control the purity and electrophoretic mobility of the protein preparations (I, II).

Crossed immunoelectrophoresis principally as described by Ganrot (1972), was used as a complement for the detection of contaminating proteins (I, II) and for comparison between the different albumins as regards heterogeneity (II).

Electroimmunoassay was performed according to Laurell (1966) in

order to determine the purity of the protein preparations (I, II) and to quantitate AFP and albumin (I, II, III), using pure AFP or albumin as standards. By use of this method it was possible to detect AFP or albumin in concentrations as low as 1 μ g/ml.

Electrophoresis in 1% agarose gel was run according to Johansson (1972) for the electrophoretic identification of proteins (I, II). This method was used in paper IV in combination with autoradiography in order to ascertain whether radioactive ligands bound to AFP or albumin.

Electrophoresis was also run in polyacrylamide gels (gradiporegel 4-26%) in order to determine the purity of the isolated protein fractions (I, II). This method is based on the separation of molecules with respect to charge and size, thereby resulting in a superior separation compared to electrophoresis in agarose.

The molecular weights of AFP (I) and albumin (II) were determined by gelfiltration in Sephadex G-200, according to the method of Andrews (1965). The isoelectric pH for AFP (I) and albumin (II) was determined by isoelectric focusing in a pH gradient built up by Ampholine carrier ampholytes (LKB). Ultracentrifugation and amino acid analysis were carried out on pure preparations of AFP and albumin (I, II).

The protein content was assayed according to Lowry et al. (1951), using bovine serum albumin fraction V as a standard (I, II, III).

Immune sera

Immune sera were produced by immunizing rabbits with protein solutions emulsified in Freund's incomplete adjuvant. The procedure is described in papers I and II.

DISCUSSION OF METHODS

When isolating protein, preparative electrophoresis in pevicone is a suitable initial step as large amounts of protein can be purified without exposure to denaturing conditions.

Affinity chromatography offers unique possibilities for protein purification. This technique utilizes the specificity of molecular interactions, and it is possible to isolate either of the components in a reversibly reacting system. The specificity between antigen and antibody is utilized in the immunosorbent techniques. It is possible to couple either antigens or antibodies to an insoluble matrix (e.g. Sepharose) and at the same time retain their specific binding activity. Using this technique it is possible to purify either antigens or antibodies. The immunosorbent technique can be used principally in two ways:

- 1) The protein to be isolated is bound to the immunosorbent column, while the contaminating proteins pass through.
- 2) The contaminating proteins are bound to the immunosorbent column, while the protein to be isolated passes through.

The proteins which are bound to the immunosorbent column can be eluated by changing the experimental conditions (e.g. pH, ionic strength) and the column can be reused several times.

The second method was used for the purification of AFP (I) and albumin (II) (affinity chromatography with ASS-sepharose) and for obtaining monospecific antibodies against AFP (I) (affinity chromatography with SS-sepharose). With access to monospecific antibodies against AFP and albumin, it was possible to isolate AFP and albumin according to the first immunosorbent technique described above.

DISCUSSION OF THE MAIN RESULTS OF THE INVESTIGATION

Isolation and characterization of AFP and albumin

The isolation and characterization of AFP and albumin are described in detail in papers I and II. The combination of preparative electrophoresis and affinity chromatography proved to be successful as pure preparations of AFP and albumin were obtained.

The degree of purity of any protein fraction is defined by the analytical methods used. The purity of the isolated AFP and albumin fractions was determined by polyacrylamide gel electrophoresis, immunological gel precipitation methods such as electroimmunoassay and by the immunization of rabbits with the isolated protein fractions.

The AFP fraction, obtained after chromatography with ASS-sepharose, was shown to be pure when tested by various immunochemical methods. However, polyacrylamide gel electrophoresis of this fraction revealed one distinct and one diffuse band. By immunizing rabbits this diffuse band was shown to be a contaminating protein. This demonstrates the importance of combining immunochemical and other biochemical methods when determining the purity of a protein fraction. Immunological methods are highly sensitive. However, the detection of a protein by use of these methods presupposes the existence of antibodies against the protein. Thus, a particular protein with poor immunogenic properties cannot be detected by immunization of an animal or by gel precipitation tests.

The biochemical data obtained for AFP and albumin are presented in papers I and II. The molecular weight as well as the sedimentation coefficients for AFP and SS-albumin were found to be almost identical. Moreover, the electrophoretic mobility for AFP and albumin was very similar. In a gel electrophoretic run at pH 8.6, AFP moves just behind albumin.

However, isoelectric focusing revealed differences in isoelectric pH between AFP and albumin. The pI value for AFP was found to be 4.61 whereas albumin from adult swine serum appeared heterogeneous, with pI values of 4.6, 4.9 and 5.5.

The amino acid composition of porcine AFP and SS-albumin showed similarities.

Thus, the present results demonstrate that AFP and albumin from the pig with respect to chemical and physicochemical properties show great similarities. These results agree with those of Grigorova et al. (1977) who reported a close analogy in physicochemical behaviour between rat serum albumin and rat AFP. Furthermore, Ruoslahti and Terry (1976) demonstrated that human AFP and serum albumin show sequence homology. However, these two proteins do not cross react immunologically in their native forms although strong cross reaction can be induced after the unfolding of their polypeptide chains with the aid of a disulphide bond reducing agent (Ruoslahti and Engvall, 1976). These results indicate that human AFP and albumin are structurally related and have a common ancestral gene (Ruoslahti and Terry, 1976).

Levels of AFP and albumin in serum from fetal, neonatal and adult pigs

The levels of AFP, albumin and total serum protein during the ontogenetic development of the pig are presented in paper III.

The concentration of AFP in the pig fetus decreases from 3 mg/ml at 6.5 cm of CR-length (corresponding to half the gestational period (Book and Bustad, 1974)) to 0.8 mg/ml at 28 cm of CR-length (near full term), whereas the albumin concentration increases from 0.2 mg/ml at 6.5 cm of CR-length to 1.0 mg/ml at 28 cm of CR-length. The total serum protein content in the fetus is approximately 20 mg/ml irrespective of gestational age. However, during neonatal development, abrupt changes

in these parameters occur. The concentration of total serum protein increases during the first day of extrauterine life from 20 to 70 mg/ml and the albumin level increases from 3 to approximately 30 mg/ml serum during the first three weeks after birth. The postnatal level of AFP shows a unique course compared to that of most other animal species, as the concentration in serum increases to a peak value of 1.02 mg/ml on the fifth day. AFP can not be detected in the serum of 8 week old piglets by electro-immunoassay.

By the aid of ^3H -leucine incorporation, the synthesis of AFP, albumin and total serum protein was examined in five neonatal pigs of different ages. This investigation showed that synthesis of AFP does occur in the pig after birth. This postnatal synthesis of AFP most probably explains the observed postnatal increase in AFP concentration. The increase in serum AFP corresponds, at the cellular level, to an increased fluorescence intensity for AFP in hepatocytes from 2-3 day old piglets compared to pig fetuses with a CR-length of 25 cm (Carlsson and Ingvarsson, 1979).

The mechanisms responsible for the observed changes in serum AFP during fetal and neonatal development are not known. We have shown that the decrease in AFP concentration from the 5th day after birth and onwards was accelerated after injections of adult swine serum (Carlsson and Ingvarsson, 1976). Similar results have also been reported for neonatal mice (Tumyan et al., 1975). Whether or not this effect is caused by a specific inhibition of AFP synthesis is under investigation.

It is obvious that an inverse relationship exists between the serum concentration of AFP and that of albumin during the ontogenetic development of the pig. Thus, AFP appears in a high and albumin in a low concentration during fetal development. In adult pigs, however, albumin dominates in serum and AFP is found only in trace amounts. Similar changes in the concentration of AFP and albumin during ontogenetic development have also been

reported for man (Nayak and Mital, 1977), rat (Nayak and Mital, 1977) and mouse (Abelev, 1971).

It seems reasonable to assume that AFP exhibits its main biological function during fetal development, whereas albumin has its dominating function in adults.

THE BIOLOGICAL FUNCTION OF AFP DURING FETAL DEVELOPMENT

The biological function of AFP during fetal development is still largely unknown. One reason for this is that research on AFP has been focused mainly on problems associated with malignant growth. During the latest years a growing interest in questions relating to the functional aspects of AFP in normally developing biological systems can be observed.

Previous studies have revealed that AFP obtained from the rat and mouse has a high binding affinity ($K_a \approx 10^8 \text{ M}^{-1}$) to 17β -estradiol and estrone (Soloff et al., 1976; Savu et al., 1977; Aussel et al., 1978). This binding appears to be specific, as related substances such as 17α -estradiol and testosterone do not react. However, AFP obtained from man, cow, rabbit, guinea pig and hamster does not show any significant estrogen binding ability (Savu et al., 1974; Swartz and Soloff, 1974; Attardi and Ruoslahti, 1977). Preliminary experiments from our laboratory indicate that porcine AFP does not bind significant amounts of 17β -estradiol (unpublished data). This discrepancy in estrogen binding ability between AFP from different animal species suggests that AFP has different functional properties in different species.

The relevance of the estrogen binding capacity is not understood. It is possible that AFP, by binding to estrogen, protects the embryo from exposure to the high estrogen levels of the mother (Savu et al., 1977). The possibility that AFP is a constituent of the estrogen-receptor in estrogen sensitive target cells as suggested by Uriel et al. (1976) has

been repudiated by other investigators (Attardi and Ruoslahti, 1977). A third proposed function of the estrogen binding ability of AFP is that AFP plays a role in the regulation of hepatocellular proliferation by binding to intracellular estrogen. This binding leads to a decreased synthesis of very low density lipoproteins thereby resulting in an increased DNA synthesis (Sell et al., 1975).

Recent studies have shown that AFP obtained from mouse and man may have an immunoregulatory role during fetal development. This statement is based on in vitro studies which showed that AFP is a potent inhibitor of mixed lymphocyte reactivity and mitogen induced lymphocyte transformation (Murgita and Tomasi, 1975b; Yachnin and Lester, 1976). Furthermore, using an in vitro plaque technique, AFP was found to have a non-cytotoxic inhibitory effect on the primary and secondary synthesis of antibodies (Murgita and Tomasi, 1975a). The mechanisms of AFP induced immunosuppression are still a matter for speculation.

It has been suggested that the immunosuppressive properties of AFP may be of importance in protecting the histoincompatible fetus from immunological attack by the maternal immune system (Murgita and Tomasi, 1975a). However, an immunosuppressive role for AFP in vivo can only be indirectly implied from the available in vitro data (Murgita and Wigzell, 1976), and in fact this proposed ability of AFP is disputed as other studies suggest that AFP may not have any biologically significant immunosuppressive function (Littman et al., 1977; Sell et al., 1977; Sheppard et al., 1977).

DOES AFP EXHIBIT FUNCTIONS SIMILAR TO THOSE OF ALBUMIN?

From the biochemical characterization of AFP and albumin it is evident that great similarities exist between these two proteins (I, II). Furthermore, the fact that their serum levels show reciprocal variations during the ontogenetic development (III) have led to speculations as to

whether AFP in the pig fetus has functions similar to those of albumin in the adult. This has also been proposed for other animal species by Abelev (1971); Ruoslahti and Terry (1976) and Sell and Becker (1978).

It is known that albumin exhibits important specific functions in the blood. It is a major reserve of amino acids and the principal osmoregulator of the blood (Peters, 1975). Furthermore, albumin transports small substances in the blood, such as ions, fatty acids, amino acids, hormones and bilirubin (Peters, 1975). It has also been shown that albumin has an affinity for drugs and dye substances (Peters, 1975). The importance of transporting small substances in the blood is partly that these are trapped within the blood when passing the kidneys, and partly that substances insoluble in blood such as fatty acids and bilirubin can be transported bound to a protein (Peters, 1975).

It is justified to ask if the low content of albumin in fetal swine serum (0.2-1 mg/ml compared to 35 mg/ml in the adult) is sufficient to carry out these functions.

In the experiments presented in paper IV, ^{14}C -labelled palmitic, stearic, oleic and arachidonic acids as well as tryptophan were added to either fetal swine serum, adult swine serum, isolated AFP or isolated albumin according to the method of Spector and Hook (1969). With the aid of gel electrophoresis and autoradiography it was possible to demonstrate that AFP as well as albumin bound the mentioned ligands independent of whether the proteins were isolated or were components of fetal or adult swine serum. These findings suggest that AFP could have a transport function in the blood of the fetus.

Further evidence for this hypothesis is that AFP derived from man has the capacity of binding dye substances (Endo et al., 1974), Cu (II) and Ni (II) ions (Aoyagi et al., 1978) and bilirubin (Aoyagi et al., 1979). Moreover, it has recently been shown that human AFP, purified under mild

conditions, contains fatty acids (Parmelee et al., 1978).

CONCLUSION AND SPECULATION

The conclusion I have drawn from papers I-IV is that AFP during the fetal development of the pig probably exhibits transport functions similar to those of albumin. This statement is supported by the following findings:

- 1) AFP and albumin derived from pig serum show similarities in chemical and physicochemical properties. (I, II).
- 2) The serum levels of AFP and albumin show reciprocal variations during the ontogenic development of the pig with the exception of the first week of extrauterine life when both AFP and albumin levels increase in serum (III).
- 3) AFP and albumin from pig serum bind tryptophan and fatty acids of different lengths and saturation (IV).

However, in order to obtain conclusive evidence it is necessary to investigate if AFP binds other types of ligands which are known to be bound to albumin, and also to determine the association constants between AFP and the various ligands.

An interesting aspect to discuss is whether there is any functional advantage in having AFP as a prenatal and albumin as a postnatal transport protein in the blood.

One possibility is that AFP and albumin are equally efficient in transporting ligands. The advantage of partly replacing albumin with AFP during fetal development may be that AFP has other important functions in the fetus that cannot be effected by albumin, such as estrogen binding and immunosuppression.

Another possibility is that differences exist between AFP and albumin in their ability to bind ligands, and that these differences have

a functional advantage. In fact, Parmelee et al. (1978) suggest that human AFP has a greater binding affinity for unsaturated fatty acids compared to human serum albumin.

It has been shown that hemoglobin, the oxygen binding protein in blood, exists in one fetal and one adult form (Darling et al., 1941). The structure of fetal hemoglobin is similar to that of adult hemoglobin except that the β chains are replaced by γ chains (Huehns et al., 1964). This difference results in a greater ability for fetal hemoglobin to bind O_2 compared to adult hemoglobin at a given pO_2 (Darling et al., 1941). Thus, the transport of O_2 across the placenta from the mother to the fetus is facilitated, this being advantageous to the fetus. After birth, the fetal form of hemoglobin disappears from blood and is replaced by the adult form (Huehns et al., 1964).

It is interesting to speculate if AFP and albumin form a protein couple operating in an analogous way to fetal and adult hemoglobin. The fetus obtains metabolites and disposes of catabolites via the placenta during pregnancy (Miller and Berndt, 1975). The mechanisms of transporting substances across the placenta are still under investigation, but it has been shown that active transport as well as diffusion of molecules does occur (Miller and Berndt, 1975).

The transport of metabolites across the placenta from the maternal to the fetal circulation should be facilitated if AFP in fetal blood exhibits a greater binding affinity for these substances than albumin in maternal blood. This would be of functional advantage, enhancing the efficiency of nutrient supplies to the fetus. Moreover, if albumin in maternal blood exhibits a greater binding affinity for catabolites than AFP in fetal blood, it implies that the transport of catabolites from the fetus to the mother is facilitated. Thus, fetal and maternal catabolites are not accumulated in the fetus.

According to the above-mentioned hypothesis, not only differences between the binding abilities of AFP and adult serum albumin must be considered, but also those of adult and fetal serum albumin. However, Huntley et al. (1977) have shown that no differences can be noted in the ligand binding abilities of fetal and adult bovine serum albumin.

Further experimental work is necessary to ascertain whether AFP is a fetal counterpart to albumin.

SUMMARY

- 1) Alpha-fetoprotein, AFP, from fetal pig serum and albumin from adult pig serum, fetal pig serum, sow's colostrum and urine from neonatal pigs were isolated by the use of preparative electrophoresis and immunosorbent techniques. The purity of the various protein fractions was ascertained by means of polyacrylamide gel electrophoresis, electroimmunoassay and by immunization of rabbits.
- 2) AFP and the various albumins were characterized with respect to molecular weight, sedimentation coefficient, isoelectric pH, amino acid composition and electrophoretic mobility. It was shown that great similarities existed between AFP and albumin in chemical and physicochemical properties.
- 3) The serum levels of AFP, albumin and total protein were estimated during the ontogenetic development of the pig. The results show the existence of an inverse relationship between the serum levels of AFP and albumin, with decreasing AFP and increasing albumin levels, except for the first week of extrauterine life when both AFP and albumin increase in serum.
- 4) By means of gel electrophoresis and autoradiography it was demonstrated that AFP and albumin bind tryptophan and fatty acids of different lengths and saturation.
- 5) The results indicate that AFP may be a fetal counterpart to adult serum albumin as regards the transport of ligands in the blood. The fact that a protein other than albumin shows affinity for several ligands in fetal blood provides a basis for a tentative hypothesis that AFP facilitates the transport of metabolites from the maternal to the fetal circulation via the placenta by having a higher affinity for these substances than albumin in maternal blood. Moreover, if maternal albumin has a higher binding affinity for catabolites than

AFP, the clearance of catabolites from fetal circulation would be improved.

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